09/156367

Copy

DOCKET NO.: L0624.70000US00

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patentee:	Ya Fang Liu
Patent No.:	6,811,992 B1
Confirmation No.:	9992
Filed:	September 17, 1998
For:	METHOD FOR IDENTIFYING JNK AND MLK INHIBITORS FOR TREATMENT OF NEUROLOGICAL CONDITIONS
Examiner:	Marianne P. Allen
Art Unit:	1631

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being placed in the United States mail with first-class postage attached, addressed to Mail Stop Certificate of Correction, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the 16th day of November, 2004.

Melissa L. Barlow Lyons

Melissa L. Barlow Lyons

Mail Stop Certificate of Correction
Commissioner For Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Transmitted herewith are the following documents:

- [X] **Request for Entrance of Certificate of Correction Under 35 U.S.C. §254 & §255**
- [X] **Certificate of Correction - Form PTO-1050**
- [X] **Copy of pertinent pages from U.S. Patent No. US 6,811,992 B1**
- [X] **Return Receipt Postcard**

If the enclosed papers are considered incomplete, the Mail Room and/or the Application Branch is respectfully requested to contact the undersigned at (617) 646-8000, Boston, Massachusetts.

A check is not enclosed. If a fee is required, the Commissioner is hereby authorized to charge Deposit Account No. 23/2825. A duplicate of this sheet is enclosed.

Respectfully submitted,
Ya Fang Liu, Patentee

By: John R. Van Amsterdam

John R. Van Amsterdam, Ph.D., Reg. No.: 40,212
Wolf, Greenfield & Sacks, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210-2206
Telephone: (617) 646-8000

Docket No.: L0624.70000US00
Date: November 16, 2004
xNDDx

DOCKET NO.: L0624.70000US00

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patentee:	Ya Fang Liu
Patent No.:	6,911,992 B1
Confirmation No.:	9992
Filed:	September 17, 1998
For:	METHOD FOR IDENTIFYING JNK AND MLK INHIBITORS FOR TREATMENT OF NEUROLOGICAL CONDITIONS
Examiner:	Marianne P. Allen
Art Unit:	1631

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being placed in the United States mail with first-class postage attached, addressed to Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the 16th day of November, 2004.

Melissa L. Barlow Lyons

Mail Stop Certificate of Correction
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR ENTRANCE OF CERTIFICATE OF CORRECTION
UNDER 35 U.S.C. §254 and §255

Sir/Madam:

Patentee respectfully requests the correction of two errors in the printing of the above-captioned patent. Specifically, the title "METHOD FOR IDENTIFYING MLK INHIBITORS FOR THE TREATMENT OF NEUROLOGICAL CONDITIONS" should be replaced with --METHOD FOR IDENTIFYING JNK AND MLK INHIBITORS FOR TREATMENT OF NEUROLOGICAL CONDITIONS--. Additionally, claim 4 has a typographical error made by the Patent Office. Please correct as follows: In column 23, line 57, "glutarnate" should be replaced with --glutamate--.

Patentee points out that the correction requested does not involve change in the patent that constitutes new matter or would require reexamination, and therefore, respectfully request that a certificate of correction be issued. Patentee encloses a copy of the pertinent pages of the issued patent with the errors highlighted. Since the error was made by the Patent Office, it is respectfully submitted that no fee is due. However, if the Examiner deems a fee necessary, the fee may be charged to Deposit Account No. 23/2825. Should any questions arise concerning the foregoing, please contact the undersigned at the telephone number listed below.

For the reasons stated above, Patentee respectfully requests entrance of the enclosed Certificate of Correction.

Respectfully submitted,
Ya Fang Liu, Patentee

By: _____
John R. Van Amsterdam, Reg. No. 40,212
Wolf, Greenfield & Sacks, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210-2211
Telephone: (617) 646-8000

Docket No. L0624.70000US00
Date: November 16, 2004
xNDD

843163.1

- 1 DEC 2004

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : US 6,811,992 B1

DATED : November 16, 2004

INVENTORS : Ya Fang Liu

It is certified that two errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the title page, item (54) should read:

METHOD FOR IDENTIFYING JNK AND MLK INHIBITORS FOR TREATMENT OF NEUROLOGICAL CONDITIONS

In the claims:

Claim 4,

In Column 23, line 57, delete "glutarnate" and replace with --glutamate--.

MAILING ADDRESS OF SENDER:

John R. Van Amsterdam
Wolf, Greenfield & Sacks, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210-2211

PATENT NO. US 6,811,992 B1

- 1 DEC 2004

Form PTO-1050843166.1

US006811992B1

(12) **United States Patent**
Liu

(10) **Patent No.: US 6,811,992 B1**
(45) **Date of Patent: Nov. 2, 2004**

(54) **METHOD FOR IDENTIFYING MLK INHIBITORS FOR THE TREATMENT OF NEUROLOGICAL CONDITIONS**

(76) Inventor: **Ya Fang Liu**, One Emerson Place, 5G, Boston, MA (US) 02114

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/156,367**

(22) Filed: **Sep. 17, 1998**

Related U.S. Application Data

(60) Provisional application No. 60/085,439, filed on May 14, 1998.

(51) **Int. Cl.**[7] **C12Q 1/00**; C12Q 1/68; C12Q 1/48; G01N 33/53; C12P 21/06

(52) **U.S. Cl.** **435/15**; 435/4; 435/6; 435/7.1; 435/69.1

(58) **Field of Search** 435/4, 15, 6, 7.1, 435/69.1

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,980,281	A		12/1990	Housey
5,385,915	A		1/1995	Buxbaum et al.
5,461,146	A		10/1995	Lewis et al.
5,468,872	A		11/1995	Glicksman et al.
5,475,110	A		12/1995	Hudkins et al.
5,516,772	A		5/1996	Glicksman et al.
5,534,426	A		7/1996	Karin et al.
5,554,523	A		9/1996	Reddy et al.
5,591,855	A		1/1997	Hudkins et al.
5,593,884	A		1/1997	Karin et al.
5,594,009	A		1/1997	Hudkins et al.
5,605,808	A		2/1997	Karin et al.
5,621,100	A		4/1997	Lewis et al.
5,621,101	A		4/1997	Lewis et al.
5,676,945	A		10/1997	Reddy et al.
5,705,511	A		1/1998	Hudkins et al.
5,741,808	A		4/1998	Lewis et al.
5,750,555	A		5/1998	Trostmann et al.
5,756,494	A		5/1998	Lewis et al.
5,817,479	A	*	10/1998	Au-Young et al. 435/69.1
5,840,509	A	*	11/1998	Ni et al.
5,854,043	A	*	12/1998	Johnson
6,060,247	A	*	5/2000	Miller et al. 435/6
6,127,401	A		10/2000	Singh et al.
6,159,948	A		12/2000	Robertson et al.
6,455,525	B1		9/2002	Singh et al.
6,514,745	B1		2/2003	Karin et al.
2002/0028815	A1		3/2002	Ator et al.
2002/0061920	A1		5/2002	Gingrich et al.
2002/0198219	A1		12/2002	Grant et al.

FOREIGN PATENT DOCUMENTS

CA	2148898	5/1995
WO	WO 93/15201	8/1993
WO	WO 94/17498	8/1994
WO	WO 95/03324	2/1995
WO	WO 95/23849	9/1995
WO	WO 99 18193	4/1999
WO	WO 99/58982	11/1999
WO	WO 00/13015	3/2000
WO	WO 00/47583	8/2000
WO	WO 02/14536	2/2002

OTHER PUBLICATIONS

Liu et al., *Society for Neuroscience Abstracts*, 23(1–2): 1909, Oct. 1997.*

Gooderough et al., *Society for Neuroscience Abstracts*, 23(1–2):1387, Oct. 1997.*

Cheung, et al., *Journal of Neuroscience Research*, 52(1):69–82, Apr. 1998.*

Yardin et al., *Neuroreport*, 9(9):2077–80, Jun. 1998.*

Yan et al., Activation of stress-activated protein kinase by MEKK1 phosphorylation of its activator SEK1, Dec. 1994, Nature, vol. 372, pp. 798–300.*

Tibbles et al., MLK-3 activates the SAPK/JNK and p378/RK pathways via SEK1 and MKK3/6, 1996, The EMBO Journal, vol. 15, no. 24, pp. 7026–7035.*

Angeles, T. et al., Enzyme-linked Immunosorbent Assay for trkA Tyrosine Kinase Activity, Analytical Biochemistry, 236: 49–55, 1996.

Bergeron et al., Inhibition of Cell Growth by Overexpression of the ZPK Gene. Biochemical and Biophysical Research Communications, 231:153–155, 1997.

Blouin et al., Cell-Specific Expression of the ZPK Gene in Adult Mouse Tissues. DNA and Cell Biology, 15: 631–642, 1996.

Davis, R.J., Human JNK3 Alpha 2 Protein Kinase (JNK3A2) mRNA. GenBank Accession No. U34819, Jul. 25, 1996.

Davis, R.J., Human JNK3 Alpha 2 Protein Kinase (JNK3A2) mRNA. GenBank Accession No. U34820, Jul. 25, 1996.

DeAizpurua et al., Expression of Mixed Lineage Kinase-1 in Pancreatic β-Cell Lines at Different Stages of Maturation and During Embryonic Pancreas Development. The Journal of Biological Chemistry, 272:16364–16373, 1997.

Diener et al., Activation of the c-Jun N-terminal kinase pathway by a novel protein kinase related to human germinal center kinase. Proc. Natl. Acad. Sci. USA, 94:9687–9692, 1997.

(List continued on next page.)

Primary Examiner—Marianne P. Allen
(74) *Attorney, Agent, or Firm*—Wolf, Greenfield & Sacks, P.C.

(57) **ABSTRACT**

The present invention describes methods for identifying compounds that inhibit JNK and MLK kinase activity as drugs for treating a mammal susceptible to or having a neurological condition. This invention also discloses methods for preventing neuronal cell death and treating neurological conditions that involve neuronal cell death, particularly neurodegenerative diseases characterized by glutamine or kainate mediated toxicity, such as Huntington's disease and Alzheimer's disease.

13 Claims, 14 Drawing Sheets

Example 8

96-Well Cell Based Assay

HN33 cells (~60% of confluence), plated on a 96-well plate, were grown in DMEM-F12 medium supplemented with 10% of fetal bovine serum (FBS). Prior to transfection, the medium was removed and cells were washed with serum-free medium once and 50 μl of DMEM-F12 medium with 1% of FBS was added. The full-length huntingtin expression plasmid containing 16 (control),48 or 89 CAG repeats or lipofectin solution were diluted with HBS first and mixed 1x1 volume and the mixture was incubated at room temperature for 15 min. Ten μl of the DNA-lipofectin mixture was added to the culture medium. A mixture of compound from a chemical library was added 6 hours after transfection. Twelve hours after transfection, additional FBS was added to the final concentration of 10%. Forty-eight hours after transfection, cells were washed with PBS once and fixed with 4% paraformaldehyde dissolved in PBS and permeabilized with 1% Triton X100 in PBS containing 1% sodium citrate. Cell were then rinsed twice with PBS containing 1% BSA and cells were incubated with 25 μl of TUNEL reaction solution for 1 hour at a 37° C. cell culture incubator. Cell were rinsed with PRS for three times and 25 μl of converter-AP solution was added and cells are returned to the incubator and incubated for 30 min. Cells were rinsed three times with PBS with 1% BSA and cells were incubated in a BCIP solution for 1–5 min at room temperature and rinsed three times with PBS and analyzed under light microscope.

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the spirit and scope of the invention as defined by the appended claims.

What is claimed is:

1. A method for assessing a compound's ability to prevent neuronal cell death, comprising:

a) contacting a compound with cultured neuronal cells having activated MLK activity, wherein activated MLK activity is selected from the group consisting of MLK1 activity, MLK2 activity, MLK3 activity, and wherein the activity is a kinase activity; and

b) determining the number of cultured neuronal cells that die; wherein a decreased number of dead cultured cells in the presence of the compound compared to the number of dead cultured neuronal cells in the absence of the compound is indicative of the compound's ability to prevent neuronal cell death.

2. The method of claim 1, wherein the neuronal cells are expressing a mutated protein selected from the group consisting of polyglutamine stretch-expanded huntingtin and C-terminal 100 amino acids of amyloid precursor protein, or the neuronal cells are treated with a neurotoxin to induce apoptosis.

3. The method of claim 2, wherein the neuronal cells are HN33 cells.

4. The method of claim 2, wherein the neurotoxin is glutamate, quinolinic acid or kainic acid.

5. The method of claim 1, wherein the neuronal cells are apoptotic neurons.

6. A method for assessing a compound's ability to prevent neuronal cell death, comprising:

a) contacting a compound with cultured neuronal cells expressing a mutated protein selected from the group consisting of polyglutamine stretch-expanded huntingtin and C-terminal 100 amino acids of amyloid precursor protein, or with neuronal cells treated with a neurotoxin to induce neuronal cell death; and

b) determining the number of cultured neuronal cells that die; wherein a decreased number of dead cultured neuronal cells in the presence of the compound compared to the number of dead cultured cells in the absence of the compound is indicative of the compound's ability to prevent neuronal cell death.

7. The method of claim 6, wherein the neuronal cells are HN33 cells.

8. A method for assessing the ability of a compound to prevent neuronal cell death, comprising:

a) contacting a compound with cultured neuronal cells having activated MLK activity, wherein the activated MLK activity is selected from the group consisting of MLK1 activity, MLK2 activity, MLK3 activity, and wherein the activity is a kinase activity;

b) contacting, in the presence of the compound, surviving cells from step (a) with an agent that induces apoptosis; and

c) comparing the level of apoptosis in the cells in the presence of the compound with the level of apoptosis in the cells in the absence of the compound; wherein the compound is a potentially useful drug for treating mammals when the level of apoptosis in the cells in the presence of the compound is less than the level of apoptosis in the cells in the absence of the compound.

9. The method of claim 8, wherein the apoptotic agent is a neurotoxin.

10. The method of claim 9, wherein the neurotoxin is glutamate, quinolinic acid or kainic acid.

11. The method of claim 8, wherein step (b) is performed by transfecting the surviving neuronal cells with nucleic acid encoding a mutated form of huntingtin or amyloid precursor protein.

12. The method of claim 8, wherein the neuronal cells are HN33 cells.

13. A method for assessing the ability of a compound to inhibit MLK activity and to prevent neuronal cell death, comprising the steps of:

a) contacting a compound with a MLK protein and a substrate therefor, wherein the MLK protein is selected from the group consisting of MLK1, MLK2, MLK3 and combinations thereof;

b) measuring the level of MLK activity, wherein the MLK activity is a kinase activity;

c) comparing the level of MLK activity in the presence of the compound with the level of MLK activity in the absence of the compound, wherein a decrease in MLK activity in the presence of the compound is indicative that the compound has an ability to inhibit MLK activity;

d) contacting the compound having an ability to inhibit MLK activity with cultured neuronal cells having activated MLK activity, wherein the activated MLK activity is kinase activity; and

e) comparing the occurrence of apoptosis in the cultured neuronal cells in the presence of the compound with the occurrence of apoptosis in the cultured neuronal cells in the absence of the compound; wherein the compound having an ability to inhibit MLK activity has the ability to prevent neuronal cell death when the occurrence of apoptosis in the cultured neuronal cells in the presence of the compound is less than the occurrence of apoptosis in the cultured neuronal cells in the absence of the compound.

* * * * *